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Research Article

Study of interleukin-6 levels in early diagnosis of neonatal sepsis

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ABSTRACT

Background: Neonatal septicemia is one of the commonest causes of neonatal mortality and morbidity. Interleukin-6 Levels appears to be one of the most promising candidate cytokine for early diagnosis of neonatal septicaemia. The aim and objectives of this study was to study the role of IL-6 levels as an early marker for diagnosis of neonatal sepsis and to compare IL-6 levels with other septic markers.
Methods: This is a hospital based prospective study to evaluate the efficacy of IL-6 as an early diagnostic marker of neonatal sepsis. Eighty neonates, delivered in the hospital, having risk factors for neonatal sepsis, along with those coming to hospital with signs and symptoms of sepsis up to 28 days of life (as study group) along with normal new-borns admitted to the postnatal ward without high risk factors (control group) were enrolled for this study.
Results: IL-6 Levels has shown maximum sensitivity of 95.83%, specificity of 87.50%, positive predictive value of 92%, negative predictive value of 93.33% and accuracy of 92.50 %. E. coli was the most common organism responsible for sepsis. CRP was reported to be highly sensitive (84.21%), and CBC was highly specific (75.00%), IT ratio has sensitivity of 62.5% and specificity of 56.25% while micro-ESR has shown sensitivity of 50.0% and specificity of 62.5%. Out of 80 cases, blood culture (BacTalert) showed growth in 48 cases in study group while two cases in control group. Thus blood culture positivity was 60%.
Conclusion: IL-6 test has maximum sensitivity as well as specificity in comparison with other septic markers. Blood culture is the gold standard for the diagnosis of septicemia. CRP is most sensitive while CBC is most specific marker in neonatal sepsis.

Keywords: Neonatal sepsis, Diagnostic markers, Mortality, IL-6 levels

INTRODUCTION

Neonatal Septicemia is the leading cause neonatal mortality and morbidity in India. It is estimated that 20% of all neonates develop sepsis[1] and is responsible for 30-50% of total neonatal death in developing countries.[2] Accurate and timely diagnosis of neonatal sepsis still remains a major challenge to the pediatricians and neonatologists. Mortality due to neonatal sepsis is preventable and if diagnosed early the outcome is better. Several indicators have been evaluated as septic screen for the early diagnosis of neonatal sepsis like CBC, CRP, micro-ESR, IT ratio, and blood culture. There is a constant search for better and ideal diagnostic marker. Ideal marker should be sensitive, specific and easily available. Its results should be immediate and reproducible. Recently various new markers are being studied such as IL-6, TNF-α, procalcitonin, G-CSF etc. IL-6 is an inducer of hepatic protein synthesis which promotes production and liberation of CRP and can be detected early when there is bacterial bloodstream invasion. It appears to be one of the most promising candidate cytokine for early diagnosis of neonatal septicaemia.[3,4]

In the present study conducted at Mahatma Gandhi Mission medical college and hospital, Navi Mumbai, IL-6 levels were assessed in normal healthy new-borns as well as new-borns with high risk factors for sepsis and the usefulness of IL-6 was evaluated as an early marker for sepsis detection and its effectiveness was compared with other septic markers.

METHODS

Study design

A prospective study design was used to evaluate the efficacy of IL-6 as an early diagnostic marker of neonatal sepsis. This is a hospital based study conducted in M.G.M medical college and hospital, Navi Mumbai.

Study period

July-2005 to August-2007

Sample size

Eighty neonates, delivered in the hospital, having risk factors for neonatal sepsis, along with those coming to hospital with signs and symptoms of sepsis up to 28 days of life (as study group) also normal new born admitted to the postnatal ward without high risk factors (Control group) were enrolled for this study. Newborns were treated with antibiotics for clinical evidence of sepsis & positive septic score as per neonatal sepsis score system (Table 1).

Table 1: Neonatal sepsis score system.[5]

Score	
Score 1	Maternal fever in 3rd trimester, instrumental delivery, intubations, exchange transfusion, LBW, outside delivery, abdominal distension, irritability, lethargy, convulsion, apnea
Score 2	Hypothermia, fever, local infection, refusal to feed, feed Intolerance, vomiting, loose stools, meconium aspiration
Score 3	LPV >12 hours, chorioamnionitis, cord erythema, foul smelling (cord)
Score 4	Sclerema, meningitis, DIC, NEC

Score 1 = risk of infection, Score 2 = need septic work up to exclude, Score 3 or more = investigate and treat

A detailed history was taken and examination was done. Following laboratory tests were done as soon as presumptive diagnosis of sepsis was made based on septic score system and on clinical grounds. All investigations were done within 24 hours of birth or at presentation before starting antibiotics like IL-6 levels, CBC, CRP with titer, micro-ESR, Immature to Total (IT) ratio, peripheral smear for toxic granules and band forms, blood culture (BacTalert), X-ray chest, CSF whenever indicated etc. Soon after birth, 1 ml of venous blood was drawn for blood culture. Also 5 ml of venous blood was collected for TLC, DLC, peripheral blood smear, micro-ESR and CRP. CRP was sent at 12 hours of life in newborns with high risk factors for sepsis. This study was approved by ethical committee of this hospital. Informed written consent was obtained from parents before entry into this study. Information of selected neonates including detailed history and clinical examination was recorded on a predesigned proforma.

Interleukin-6 levels determination[4]

1 ml blood was collected in plain bulb and serum was tested by chemiluminescent immunometric technique in IMMULITE Machine1000 (Table 2). IMMULITE 1000 IL-6 is a solid phase, enzyme labelled, chemiluminescent sequential immunometric assay.

The use of an ultracentrifuge is recommended to clear lipemic samples. Volume required - 100 µL serum, EDTA or heparinized plasma (Sample cup must contain at least 250 µL more than the total volume required).

Interpretation[6,7]

Table 2: Interpretation guide for immune monitoring.

Parameter	Group	Concentration	Interpretation guide
IL-6	Low level	<100 pg/ml	Infection unlikely Patient may be tested again if clinical sign persists
	High level	>100 pg/ml	Patient most likely needs treatment IL-6 levels correlate with the severity of the disease

Data analysis

Data was collected, classified, tabulated and analyzed. Tests of significance were applied at appropriate places and interpretation was done accordingly.

To evaluate the difference between the categories, McNemar Chi square test was used as a test of significance. A P value of less than 0.05 was considered statistically significant.

RESULTS

Among 40 babies of study group, 25 (62.50%) has IL-6 Test positive. Statistical analysis of IL-6 levels concentration >100 pg/ml in this study of 40 cases (study group) yielded a sensitivity of 95.83% and specificity of 87.50% whereas Positive predictive value and the negative predictive value is 92% and 93.33% respectively.

Neonatal mortality is seen in 4 cases (5%) of the total 80 cases. Of these 4 cases, all cases have shown elevated IL-6 levels. Hence, strongly elevated IL-6 levels in this study have found to be associated with bad prognosis as indicated by death.

The result of the present study appears to emphasize that serum IL-6 levels increase to a significant level in the patients having bacterial septicaemia. The level of rise depends upon the severity of the sepsis.

DISCUSSION

Of total of 80 cases, with risk factor and clinical signs and symptoms of sepsis (40 cases as study group) and normal healthy new-borns without risk factors (40 cases as control group). The study group consists of 28 males (70%) and 12 females (30%) while control group consists of 21 males (52.50%) and 19 females (47.50%). Among 40 babies of study group, 24 (60%) are blood culture (BacTalert) positive and 16 (40%) are blood culture (BacTalert) negative while in control group, 1 (2.50%) is blood culture (BacTalert) positive and 39 (97.50%) are blood culture (BacTalert) negative (Figure 1).

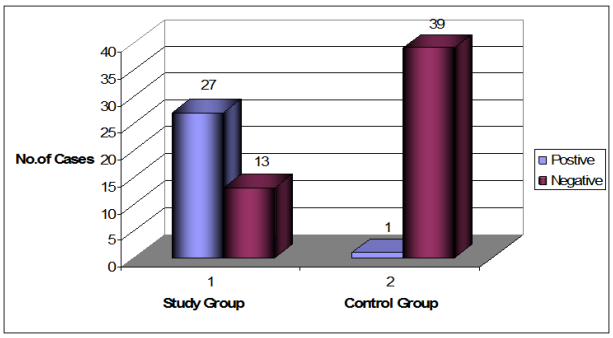

Figure 1: Showing distribution of cases according to blood culture.

In study group, E-coli comprised the maximum number of cases accounting for sepsis i.e. 7 (17.5%) followed by 5 cases (12.5%) of Acinetobacter baumanii, 5 cases (12.5%) of Klebsiella pneumoniae, 2 cases (5%) each for Citrobacter and Staphylococcus aureus and 1 case (2.5%) has shown Pseudomonous sp., Burkholderia cepacia and Fungus while no growth in 16 (40%) cases. In control group, only 1 case (2.5%) shows growth of Acinetobacter baumanii and 39 cases (97.5%) are sterile (Figure 2).

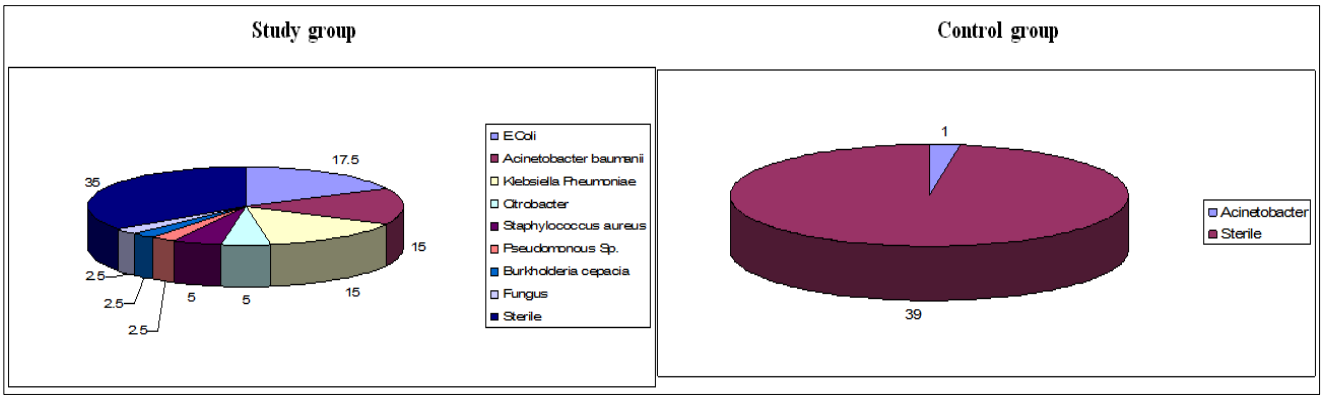

Figure 2: Showing distribution of cases according to microbiological growth on blood culture.

Bhargava et al.[8] noted in their study that the incidence of E. coli as the causative organism of neonatal sepsis was 45%. Mirf et al.[9] in their study of 50 cases also showed similar results. McCraken,[10] Faridi and Gupta,[11] Kumar GD et al.[12] have also reported that gram negative septicemia is more common than gram positive septicemia.

Out of 40 cases in study group, CBC is abnormal in 13 cases (32.5%), Blood culture (BacTalert) was positive in 24 cases (60%) and 4 cases (10%) has CBC abnormal with sterile blood culture. In this study CBC had low sensitivity (37.50%) and high specificity (75.00%). Chan and Ho[13] revealed in their study that abnormal CBC had the lowest sensitivity and PPV while abnormal ANC had the lowest specificity and NPV among them (Figure 3).

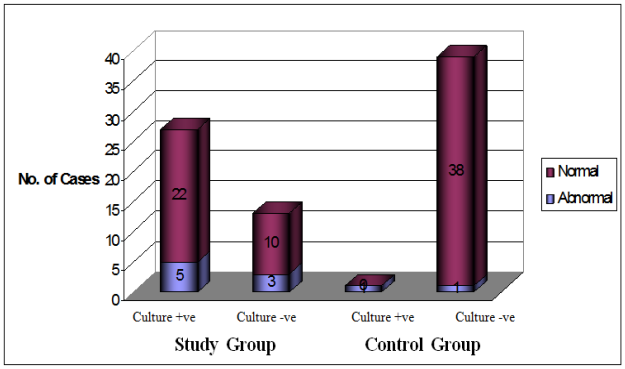

Figure 3: Showing distribution of cases according to CBC.

Out of 40 cases in study group, CRP is reactive in 31 cases (77.5%), Blood culture is positive in 24 cases (60%) and 15 cases (37.5%) are having CRP reactive with sterile blood culture. In this study CRP was reported most sensitive (84.21%) but low specific (28.57%), Franz AR et al.[14] showed that there is generally a delay of up to 24 hours between onset of symptoms of infection and a rise in serum CRP. Sensitivity of the test at presentation is only 40% that is, 60% of subsequently proven sepsis episodes will have a normal initial CRP (Figure 4).

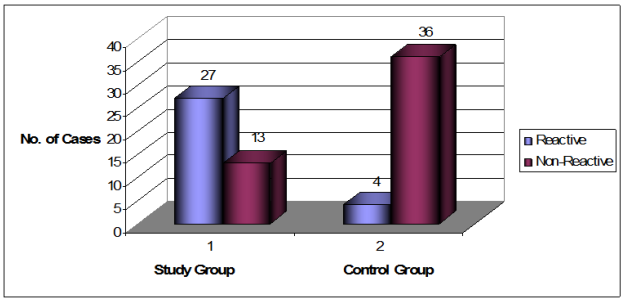

Figure 4: Showing distribution of cases according to CRP.

Out of 40 cases in study group, IT Ratio is abnormal in 22 cases (55%), Blood Culture is positive in 24 cases (60%) and 7 cases (17.5%) are having IT Ratio abnormal with sterile blood culture. In our study IT ratio was reported NPV of 50.0%. Ghosh et al.[15] studied 103 high risk neonates having predisposing perinatal factors or clinical suspicion of sepsis and found that an abnormal Immature to Total neutrophil (IT) ratio were the most sensitive indicators in identifying neonates with sepsis showing high negative predictive value over 94% (Figure 5).

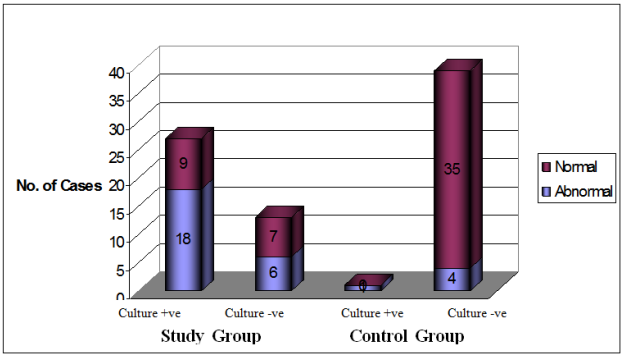

Figure 5: Showing distribution of cases according to IT ratio.

Out of 40 cases in study group, Micro-ESR is abnormal in 18 cases (45%), Blood Culture is positive in 24 cases (60%) and 6 cases (15%) has Micro ESR abnormal with sterile blood culture. In our study Micro-ESR has shown sensitivity of 50.0%, specificity of 62.5. K. K. Diwakar and, Rosul G[16] studied on 114 term neonates for early neonatal sepsis. The sensitivity and specificity of the 'revised' micro-ESR was 62.5% and 60.9% respectively in diagnosing culture proven sepsis (Figure 6).

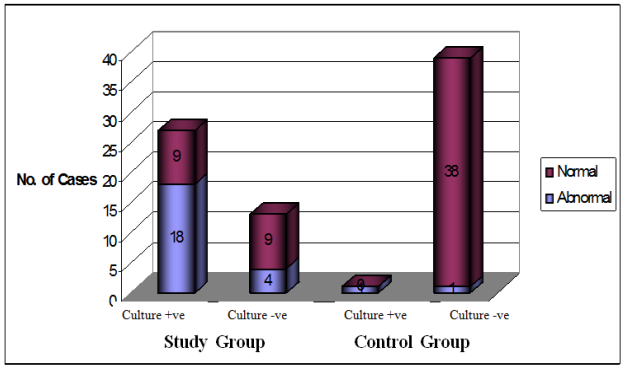

Figure 6: Showing distribution of cases according to micro-ESR.

Among 40 babies of study group, 25 (62.50%) has IL-6 Test positive and 15 (37.50%) has IL-6 Test negative while in control group, 3 (7.5%) has IL-6 Test positive and 37 (92.5%) has IL-6 Test negative. In present study, neonatal mortality is seen in 4 cases (5%) of the total 80 cases. Of these 4 cases, all cases have shown elevated IL-6 levels. Hence, strongly elevated IL-6 levels in this study have found to be associated with bad prognosis as indicated by death. Statistical analysis of IL-6 levels concentration >100 pg/ml in this study of 40 cases (study group) yielded a sensitivity of 95.83% and specificity of 87.50% whereas Positive predictive value and the Negative predictive value is 92% and 93.33% respectively. Many studies have also reported similar results. The recent study of IL-6 in early neonatal sepsis by Silveira et al.[17] using normal new born as controls, showed similar sensitivity of 96%. Recently, Silveira and Procianoy[18] reported that IL-6 and TNF-α are likely candidate cytokines for use in early diagnosis of neonatal sepsis (Figure 7).

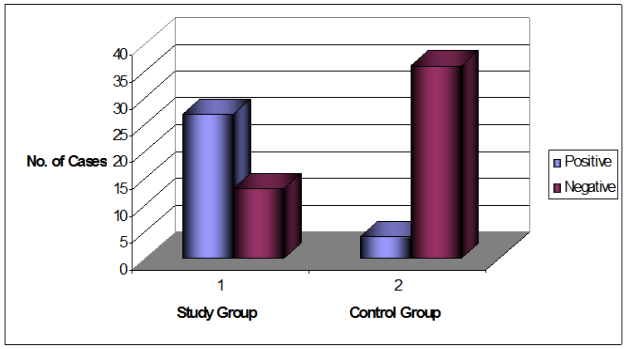

Figure 7: Showing distribution of cases according to IL-6 assay.

Out of 40 cases in study group, blood culture is positive in 24 cases (60%), IL-6 is positive in 25 cases (62.5%) and 2 cases (5%) have IL-6 positive with sterile blood culture (Figure 8).

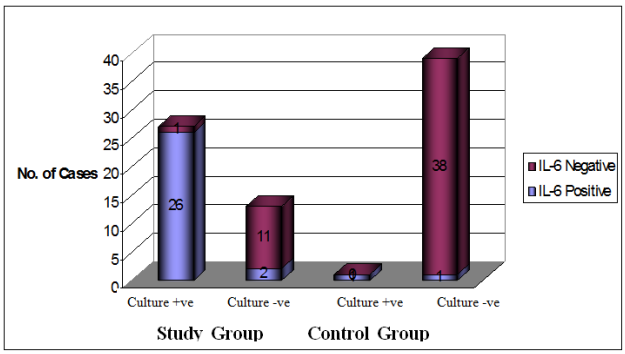

Figure 8: Showing IL-6 levels in sepsis.

Same group of patients are tested with both Blood Culture (BacTalert) as well as IL-6 levels. Therefore McNemar's (ChiSquare) test is used to evaluate whether results of these tests vary significantly from each other. It is observed that the results of both tests are not statistically significant from each other with $x^2 = 0.083$, P value equal to 1.00 and degree of freedom equal to 1.

There is no statistically significant difference between Blood Culture (BacTalert) and IL-6 assay (P = 1). Hence use of IL-6 levels for early diagnosis of neonatal sepsis can be justified (Table 3).

Table 3: IL-6 parameters: sensitivity, specificity, PPV, NPV and accuracy.

IL-6 parameters	
Sensitivity	95.83%
Specificity	87.50%
Positive predictive value (PPV)	92.00%
Negative predictive value (NPV)	93.33%
Accuracy	92.50%

Chi-square (X^2) = 0.083, Degree of freedom = 1, P value = 1.00

Thus, IL-6 test has maximum sensitivity as well as specificity in comparison with other septic markers in early detection of neonatal sepsis (Table 4).

Table 4: Comparative parameters: sensitivity, specificity, PPV, NPV and accuracy.

Tests	Sensitivity	Specificity	PPV	NPV	Accuracy
CBC	37.50%	75.00%	69.23%	44.44%	52.50%
Micro-ESR	50.00%	62.50%	66.67%	45.45%	55.00%
IT ratio	62.50%	56.25%	68.18%	50.00%	60.00%
CRP	84.21%	28.57%	51.61%	66.67%	55.00%
IL-6 assay	95.83%	87.50%	92.00%	93.33%	92.50%

Hence, it can be concluded that IL-6 concentration increases to significant level in the patient having bacterial septicaemia. IL-6 test has maximum sensitivity as well as specificity in comparison with other septic markers. Therefore, IL-6 levels can be used as an early diagnostic marker for neonatal sepsis.

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Ethical approval: The study was approved by the hospital ethics committee (IRB)

REFERENCES

1. M. Jeeva Sankar, Ramesh Agarwal, Ashok K. Deorari, Vinod K. Paul. Sepsis in the new-born. In: M. Jeeva Sankar, Ramesh Agarwal, Ashok K. Deorari, Vinod K. Paul, eds. AIIMS - NICU Protocols. New Delhi: AIIMS; 2008: 3.
2. Siegel J, McCracken G. Sepsis Neonatarum. N Engl J Med. 1981;304(11):642-7.
3. Bhartiya D, Kapadia C, Sanghvi K, Singh H, Kelkar R, Merchant R. Preliminary studies on IL-6 levels in healthy and septic Indian neonates. Indian Pediatr. 2000;37:1361-7.
4. Saunders BM, Liu Z, Zhan Y, Cheers C. Interleukin-6 production during chronic experimental infection. Immunol Cell Biol. 1993;71:275-80.
5. Gupte S. Neonatal sepsis score system. In: Gupte S, eds. The Short Text Book of Pediatrics. 9th ed. New Delhi: Jaypee Brothers; 2001: 559-561.
6. Milenia Biotec. Interpretation guide for immune monitoring, 2011. Available at: http://anna-med.co.uk/Sepsis_Neonatal.pdf.
7. de Bont E, Martens A, van Raan J, Samson G, Fetter WP, Okken A, et al. Tumor necrosis factor-alpha, interleukin-1 beta and interleukin-6 plasma levels in neonatal sepsis. Pediatr Res. 1993;33:380-3.
8. Bhargav SK, Gupta A, Bhargav M. Bacterial Infections in new-born. Newer Devel Pediatr Res. 1977;1:11.
9. Mirf Amans, Khan SR. Neonatal sepsis: a review with study of 50 cases. J Trop Pediatr. 1987;33(3):131-5.

10. McCraken, Shinefield. Changes in the pattern of neonatal septicemia and meningitis. Am J Dis Child. 1966;112:33.
11. Faridi MMA, Gupta P, Bhargava SK. Chest radiograph in neonatal sepsis. Indian Pediatr. 1972;29:871.
12. Kumar GD, Ramachandran VG, Gupta P. Bacteriological analysis of blood culture isolates from neonate in tertiary care hospital in India. J Health Popul Nutr. 2002;20(4):343-7.
13. Chan DK, Ho LY. Usefulness of C-reactive protein in the diagnosis of neonatal sepsis. Singap Med J. 1997;38(6):252-5.
14. Franz AR, Steinbach G, Kron M, Pohlandt F. Reduction of unnecessary antibiotic therapy in new-born infants using interleukin-8 and C-reactive protein as markers of bacterial infections. Pediatrics. 1999;104:447-53.
15. Ghosh S, Mittal M, Jaganathan G. Early diagnosis of neonatal sepsis using a hematological scoring system. Indian J Med Sci. 2000;54(9):495-500.
16. Diwakar KK, Rosul G. Revised look at micro-erythrocyte sedimentation rate in neonates. Indian Pediatr. 1999;36:703-5.
17. Silveira RC, Procianoy RS. Evaluation of interleukin-6, tumour necrosis factor-a and interleukin-1 for early diagnosis of neonatal sepsis. Acta Pediatr. 1999;88:647-50.
18. Renato S. Procianoy, Rita C. Silveira. The role of sample collection timing on interleukin-6 levels in early-onset neonatal sepsis. J Pediatr (Rio J). 2004;80(5):407-10.
19. Milenia Biotec. Sepsis?, 2005. Available at: http://anna-med.co.uk/Sepsis_Neonatal.pdf.

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